BOTH POLYGLYCOSYLCERAMIDES AND POLYGLYCOSYLPEPTIDES ARE UNBRANCHED IN i ERYTHROCYTES

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1. Introduction

Polyglycosylceramides are highly complex, multibranched glycosphingolipids which exhibit ABH and I antigenic activity [1]. They contribute to ~15% of the total ABH blood group activity of human erythrocytes [2–5]. About 80% of the activity is provided by glycoproteins most of which are of the *N*-glycosidic type. The glycoproteins contain polyglycosylchains which are similar in structure to those of polyglycosylceramides [6,7]. They were isolated from pronase digests of erythrocyte membranes and variously designated polyglycosylpeptides [6] or erythroglycan [7]. Polyglycosylpeptides are probably derived from band 3 and band 4.5 glycoproteins [4]. We had established that polyglycosylceramides are largely unbranched in erythrocytes of a rare i phenotype [8]. We now show that the impaired branching is also a feature of i erythrocyte polyglycosylpeptides.

2. Materials and methods

Erythrocytes with i phenotype (200 ml) were obtained from a blood group AB individual (M.T.). The erythrocytes totally lacked I^D component of I antigen. The I^F component was not depressed. The nomenclature is as in [9]. Control I erythrocytes were obtained from a type B blood donor. Polyglycosylceramides were isolated as in [8] while polyglycosylpeptides as in [7]. The starting material was erythrocyte stroma prepared as in [8].

The isolated materials were analysed for carbohydrate constituents as in [8]. Sphingosine was determined as in [10]. The number of branching points was estimated on the basis of 2,4-di-*O*-methylgalactose contents as determined in the methylated and acetolysed samples [8]. Hemagglutination inhibition assays were done as in [8] using blood group O erythrocytes as indicator cells. Carrier auxilliary lipid was not added. Some of the anti-I and anti-i serums employed in this study were used in [8] for measurements of Ii activities of polyglycosylceramides isolated from cord, adult and i erythrocytes.

3. Results

Chemical compositions of polyglycosylceramides and of polyglycosylpeptides isolated from i and I erythrocytes are shown in table 1. The molar ratios of carbohydrates in polyglycosylpeptides were calculated on the assumption that there are 3 mannose residues/mol [6,7]. A reduction of yields and molecular complexity of the two kinds of glycoconjugates from i erythrocytes is evident. Results of the examination of polyglycosylceramides and polyglycosylpeptides by methylation technique is presented in table 2. Owing to small amounts of material available we did not attempt to quantitate methyl ethers of mannose. The most conspicuous differences between glycoconjugates from i and I erythrocytes were in 2,4-di-*O*-methylgalactose contents which were very much reduced in glycoconjugates from i erythrocytes.

Table 3 shows I and i activities of the glycoconjugates as determined by hemagglutination inhibition technique. Clearly the I activity of polyglycosylpeptides from I erythrocytes is 10-fold lower than that of polyglycosylceramides. Polyglycosylpeptides from i erythrocytes were inactive or only slightly active

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Table 1
Yields and approximate molar ratios of carbohydrates and sphingosine in polyglycosylceramides and polyglycosylpeptides from I and i erythrocytes

	Polyglycosyl-ceramides from i cells	Polyglycosyl-peptides from i cells	Polyglycosyl-peptides from I cells
Yield mg/100 ml of erythrocytes	0.33	0.75	3.5
Fucose	1.3	2.0	3.1
Galactose	6.0	16.5	25.1
Glucose	1.0	trace	2.0[a]
Mannose	–	3.0	3.0
N-Acetylglucosamine	4.5	12.9	24.6
N-Acetylgalactosamine	0.4	2.3	–
Sialic acid	n.d.	3.0	2.4
Sphingosine	0.84	n.d.	–
Sum of carbohydrate residues (without sialic acid)	13.5	36.7	55.8

[a] Possibly a contaminant from Sephadex columns;
n.d., not determined; – not detectable

Results for polyglycosylceramides are given assuming that glucose is 1.0

Table 2
Approximate molar ratios of the methylated sugar derivatives from polyglycosylceramides and polyglycosylpeptides of I and i erythrocytes

	Polyglycosyl-ceramides from i cells	Polyglycosyl-peptides from i cells	Polyglycosyl-peptides from I cells
2,3,4-*O*-Me_3FucOH	1.2	2.0	3.0
2,3,4,6-*O*-Me_3GalOH	0.4	0.7	4.5
2,4,6-*O*-Me_3GalOH	3.4	12.6	10.5
2,3,4-*O*-Me_3GalOH	–	–	1.2
3,4,6-*O*-Me_3GalOH	0.6	–	–
2,4-*O*-Me_2GalOH	0.8	0.7	9.1
4,6-*O*-Me_2GalOH	1.0	2.5	1.5
2,3,6-*O*-Me_3GlcOH	1.0	–	–
3,6-*O*-Me_2GlcNAcMeOH	4.5	12.9	24.6
3,4,6-*O*-Me_3GalNAcMeOH	0.4	2.3	–

Calculations were made on the assumption that 3,6-*O*-Me_2GlcNAcMeOH is equivalent to the total *N*-acetylglucosamine as determined by alditol acetate technique. Derivatives of mannose are not shown

Table 3
I and i activities of polyglycosylceramides and polyglycosylpeptides

Substance and source	Serum			
	anti-I^{D}Baj	anti-I^{D}Szum	anti-I^{F}Zaw	anti-i St
Polyglycosylceramides from pooled I (B) erythrocytes	72	36	144	~2300
Polyglycosylpeptides from I (B) erythrocytes	700	700	700	>2800
Polyglycosylpeptides from i erythrocytes	>830	>830	~830	103.7

~ incomplete inhibition

Results are expressed as minimum amount of substances in μg which inhibit hemagglutination

with anti-I serums but inhibited anti-i serum St. The same anti-i serum was shown in [8] to react with polyglycosylceramides isolated from i erythrocytes of a different donor Dr. The antiserum is only partially inhibited by polyglycosylceramides from pooled I erythrocytes. Polyglycosylceramides from i erythrocytes of the donor M.T. could not be assayed for serological activity due to a limited amount of the material.

4. Discussion

Yields, molar ratios of sugars and methylation data for polyglycosylceramides obtained from i erythrocytes of the donor M.T. are practically identical to those reported for i erythrocytes of the donor Dr [8]. The values are significantly different from those for 'normal' polyglycosylceramides from I erythrocytes. The latter have an av. 30 sugar residues/mol and ~4–5 branching points [1,8]. The yield amounts to 1.2 ± 0.27 mg/100 ml erythrocytes. Data for polyglycosylpeptides from i erythrocytes are reported for the first time. In [8] we had shown only that glycoproteins from i erythrocytes that are labelled with *N*-[^{14}C]acetylgalactosamine with the aid of *A* enzyme (EC 2.4.1.40) have reduced molecular weights as judged by SDS gel electrophoresis [8]. These data show that polyglycosylpeptides from i erythrocytes have practically straight saccharide chains. In contrast there are 9 branching points in polyglycosylpeptides isolated from control I erythrocytes. The latter value compares favorably with the number of 8.5 branching points reported for erythroglycon [7]. These results are in keeping with our hypothesis that i phenotype results from a deficiency of the biosynthetic process which leads to the formation of GlcNAc 1 → 6 Gal sequence [1]. The sequence should initiate most of side chains in polyglycosylceramides [11]. It would be interesting to learn whether the deficient branching of glycoconjugates in individuals of i phenotype is restricted to erythrocytes or is manifested also in other types of cells.

We report for the first time direct measurements of Ii activities of polyglycosylpeptides. The low I activity of polyglycosylpeptides from I erythrocytes relative to that of polyglycosylceramides might be attributed to the fact that the latter form micells in aqueous solution [1,12,13] and thus exhibit enhanced activity in hemagglutination inhibition tests [14]. Another factor which could contribute to the higher activity of polyglycosylceramides is that they are obtained from pooled blood of at least 20 donors.

The absence of I activity and the presence of i activity in unbranched polyglycosylpeptides from i erythrocytes lend further support to the concept that anti-I antibodies recognize the repeating structure galactosyl β(1 → 4)-2-deoxy-2-acetamidoglucosyl β(1 → 3). Anti-I antibodies should on the other hand react with the same structure but branched at carbon atom 6 of galactose with *N*-acetylglucosamine [8,15,16].

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